Whitefly-active metabolites of imidacloprid: biological efficacy and translocation in cotton plants†

Ralf Nauen,[1]* Udo Reckmann,[2] Stefan Armborst,[2] Hans-Peter Stupp[3] and Alfred Elbert[1]

[1] *Bayer AG, Agrochemicals Division, Research Insecticides, Institute of Insect Control, D-51368 Leverkusen, Germany*
[2] *Bayer AG, Agrochemicals Division, Formulation Development, D-51368 Leverkusen, Germany*
[3] *Bayer AG, Agrochemicals Division, Chemical Technology and Analytical Services, D-51368 Leverkusen, Germany*

Abstract: The chloronicotinyl insecticide imidacloprid is widely used in soil application, seed treatment and as a foliar spray. Its systemic properties are well-known. It is more or less completely metabolised, depending on the method of application, plant species and time. Some of the metabolites that arise are active against different aphid species, as shown earlier, with the imidazoline derivative (olefine metabolite) more active in oral ingestion bioassays than the parent compound itself.

In the present work, we demonstrate that the olefine metabolite and two hydroxy metabolites of imidacloprid are also active against the cotton whitefly, *Bemisia tabaci*, in oral ingestion bioassays (sachet test). The 4-hydroxy metabolite is as active as imidacloprid and the olefine compound *c*10 times more active. The two hydroxy metabolites were also active against biotypes from Almeria, Spain and a B-type strain from California. The physicochemical properties of the metabolites were determined and compared with those of imidacloprid. Lower log *P* values were found for all metabolites investigated. Interestingly, the olefine metabolite was the only compound with an acidic proton (pKa 7.2) under neutral pH conditions, suggesting that it could be more phloem-mobile. In order to investigate this hypothesis we compared the translocation of the radiolabelled olefine metabolite after foliar application with the movement of radiolabelled imidacloprid in cotton plants, but no major differences were found in acropetal or basipetal translocation.

Keywords: *Bemisia tabaci;* imidacloprid; metabolites; cotton

1 INTRODUCTION

The chloronicotinyl insecticide imidacloprid was launched in 1991. It is particularly active against a range of homopteran pest species, including those already resistant to conventional insecticides.[1–3] Imidacloprid acts agonistically on insect nicotinic acetylcholine receptors, as do all other compounds belonging to the chloronicotinyls or neonicotinoids.[4,5] Imidacloprid is used in seed treatments and soil and foliar applications.[6] It is transported mainly in the xylem in both mono- and di-cotyledonous crop species.[7,8] The systemic properties of imidacloprid allow it to become evenly distributed in the young, growing plant. After spray application, most of the residue on the leaf surface consists of unchanged parent compound, whereas the imidacloprid applied by soil application or seed treatment is metabolised more or less completely, depending on plant species and time.[9,10] It has been shown recently that some of the metabolites exhibit insecticidal potency against different aphid species.[11] In particular, the olefine metabolite is at least 10 times more active than imidacloprid, which may influence the residual insecticidal activity in imidacloprid-treated plants,[11,12] despite its relatively low concentration.[9,13]

The objective of the present study was to elucidate the effects of some selected metabolites of imidacloprid that are produced in the plant against different strains of the cotton whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). We also investigated the translocation behaviour of imidacloprid and its olefine metabolite in cotton plants after foliar application. This was of particular interest because the olefine metabolite of imidacloprid exhibits an acidic proton providing a pKa value

* Correspondence to: Ralf Nauen, Bayer AG, Agrochemicals Division, Research Insecticides, Institute of Insect Control, D-51368 Leverkusen, Germany
E-mail: RALF.NAUEN.RN@bayer-ag.de
† Based on poster presentations at the 9th International Congress of Pesticide Chemistry, organised by the International Union of Pure and Applied Chemistry (IUPAC), and held in London, UK, 2–7 August 1998.
(*Received 26 June 1998; revised version received 22 September 1998; accepted 23 October 1998*)

which should in theory improve phloem-mobility.

2 MATERIAL AND METHODS

2.1 Compounds

Unlabelled imidacloprid was technical grade of the highest purity available. The imidacloprid metabolites (**1**–**5**; Fig 1) were also of the highest purity available, ie at least 97%. Imidacloprid and selected metabolites were provided in-house (Bayer AG, Leverkusen, Germany). All other chemicals and organic solvents used were of analytical grade. Stock solutions of imidacloprid and its metabolites for testing biological efficacy were prepared in acetone, and diluted with sucrose solutions (150 g litre^{-1}).

[*Pyridinyl*-^{14}C]imidacloprid had a radiochemical purity of 98% determined by TLC and was used at a specific activity of 3.85 MBq mg^{-1}. The [^{14}C]-olefine metabolite **1** of imidacloprid was provided in-house and had a radiochemical purity of > 95% and a specific activity of 3.85 MBq mg^{-1}. Radioactive compounds were diluted with acetone + water (1 + 1, by volume) to give a stock solution with a concentration of 100 g AI litre^{-1}. Prior to application, the stock solution was further diluted with water containing the organosilicone surfactant Silwet L-77 (Witco/OSI) resulting in a pseudo-formulation with a concentration of 0.5 g AI litre^{-1}. The final concentration of Silwet L-77 was 1 g litre^{-1}.

2.2 Whiteflies

All strains of *B. tabaci* were kindly provided by Dr Matt Cahill, IACR Rothamsted, Harpenden, UK. SUD-S was an insecticide-susceptible strain originally derived from cotton in Sudan and reared in the laboratory since 1978. The two Spanish strains from Almeria, Spain, ALM-2 and LMPA-2, were collected from cucumber in 1994 and melon in 1995, respectively. Both strains were somewhat insensitive to imidacloprid, and highly resistant to conventional insecticides such as organophosphates and carbamates.[14–16] CAL-1 was a typical B-strain from California, USA, collected from cotton in 1995. All strains were maintained on cotton plants (Cocker 312) at 25(± 1)°C, 60% RH and a photoperiod of L : D 16 : 8 h.

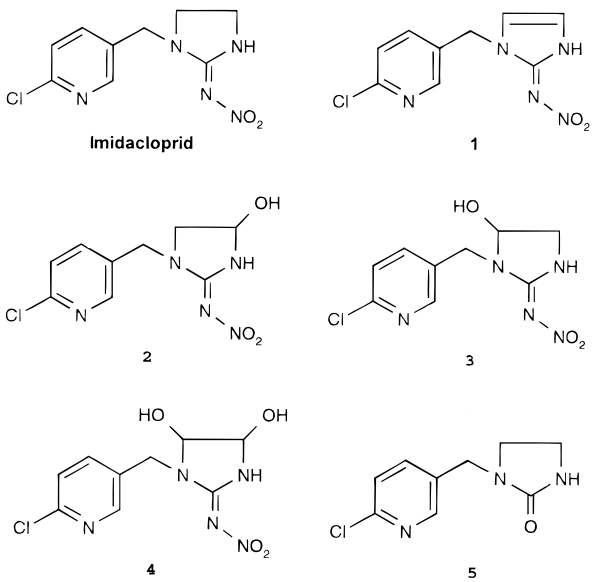

Figure 1. Chemical structures of imidacloprid and some selected metabolites.

2.3 Bioassays

2.3.1 Oral ingestion bioassay (sachet test)

The procedure for testing imidacloprid and its metabolites in a feeding bioassay using artificial double membranes has already been published in relation to resistance determination in aphids and whiteflies.[2,15] Each sachet (28 mm in diameter) consisted of two layers of stretched Parafilm enclosing 0.4 ml of a 150 g litre^{-1} sucrose solution and different concentrations of test compounds. Adult whiteflies were removed from a rearing cage, anaesthetised briefly with carbon dioxide and spread onto a black-cloth-covered ice-brick. A fine brush was used to place 20 adult females per concentration in appropriate containers which were then sealed with the corresponding double membrane. The total number of living insects per unit was scored after their recovery from narcosis. A piece of yellow cellophane was placed over the double membrane to enhance feeding activity. For each bioassay, five to six concentrations were tested, with at least three replicates. Percentage insect mortality was assessed after 48 h at 21°C and 50% RH.

2.4 Uptake and translocation experiments

2.4.1 Plant material

Cotton plants (*Gossypium hirsutum* L., cv. DPL 5415) were grown to the two-to-three-leaf stage under glasshouse conditions (temperature: day 22°C, night 22°C, light conditions: 12 h photoperiod of daylight supplemented by HQL lamps, RH 70%).

2.4.2 Application and experimental conditions

Five 2-µl droplets of the pseudo-formulation were applied to the lower third of the first fully expanded leaf. Six replicate plants were treated with radiolabelled imidacloprid and six with ^{14}C-olefine metabolite. Three of the five droplets were placed on the major veins of the leaf. A barrier of silicone grease around the site of application was used to prevent direct contamination of other plant parts. Plants were incubated for 26 h in the glasshouse (temperature 20°C day, 16°C night, 60% RH, and 16 h photoperiod with daylight supplemented by HQI-T lamps).

2.4.3 Extraction and measurement of [^{14}C]imidacloprid and metabolites

The zone of application, including the silicone grease barrier, was excised from each plant. The labelled compound that had not been taken up into the leaf was removed with acetonitrile (5 ml) and three aliquots were taken to measure the radioactivity in this extract by liquid scintillation counting (LSC). The

roots of treated plants were rinsed with water to remove soil. Three plants from each treatment were divided into three fractions; the zone of application; the part of the treated leaf above the application (corresponding to acropetal translocation); and the remainder of the plant (corresponding to phloem translocation). After measuring fresh weight (0.2–6.4 g) the three respective fractions from each plant were pooled, chopped and homogenised with pestle and mortar in liquid nitrogen. Homogenised plant fractions were extracted with methanol + water (80 + 20, by volume; 3 × 20–60 ml, depending on the fresh weight) and radioactivity was measured by LSC (scintillation fluid Quicksafe A, Zinsser Analytics, LS-Counter LKB 1219 Rackbeta) in three aliquots of the combined extracts. Extracts were filtered (nitrocellulose, 0.45 μm) and the radioactivity in the solid phase was determined after combustion in an oxidiser. The methanol was then evaporated from the filtrate in a rotary evaporator at a bath temperature of 40 °C. The remaining water phase (20 ml) was extracted with dichloromethane (3 × 50 ml), the combined extracts were evaporated to dryness in a rotary evaporator and the residue was redissolved in dichloromethane (5 ml). It was known from control experiments that both imidacloprid and the olefinic metabolite **1** were extracted into the dichloromethane phase. Between experiments all extracts were stored in a freezer at − 20°C.

Aliquots (4–30 Bq) of the dichloromethane phase and of the fraction that had been washed off the leaf surface were applied to thin-layer chromatography plates (TLC, pre-coated silica gel 60 F-254, 0.2 mm, Merck Co.). The plates were developed using two different solvent systems: (A) dichloromethane + methanol (90 + 4, by volume) and (B) ethyl acetate + 2-propanol + water (65 + 23 + 12, by volume). After drying, the plates were analysed on a bio-imaging analyser (BAS 2000, Fuji Co, software for quantification Tina version 2.09g, Raytest Co). Peaks were identified by co-chromatography with labelled reference compounds.

The three remaining plants were dried and autoradiographs were prepared on a bio-imaging analyser (BAS 2000, Fuji Co, three days' exposure). To avoid over-exposure, the site of application was exposed for a shorter time (6 h) on a separate imaging plate. Subsequently, total radioactivity was measured from the different zones of the plants (ie the part of the treated leaf above the site of application, the site of application, the stem, cotyledons, second leaf, shoot tip and roots) after combustion in an oxidiser (OX-300, Harvey Instrument Corporation). The liberated [^{14}C]carbon dioxide was trapped in a scintillation cocktail (®Carbosorb, Packard Co).

2.5 Determination of physicochemical properties

2.5.1 log $P_{o/w}$ values

Log $P_{o/w}$ values were determined using high pressure liquid chromatography. Compounds were eluted from reverse-phase columns by gradient elution (aqueous phosphoric acid 1 g litre^{-1}: acetonitrile) using 2-alkanones as a calibration reference.[17]

2.5.2 pK_a values

The dissociation constants of imidacloprid and its metabolites were measured by capillary electrophoresis using six different buffer systems between pH 2.3 and pH 11.6 according to Cleveland *et al.*[18]

2.5.3 Stability in solution: half life

The half life of a substance (0.05 g litre^{-1} in buffer + acetonitrile, 1 + 1, by volume) was defined as the time taken for 50% of it to degrade at 50°C. Buffers (0.05 M) were used to provide controlled pH conditions (pH 7: phosphate buffer; pH 9: borax/hydrochloric acid buffer).

2.6 Calculations

LC_{50} values and their 95% confidence limits for all bioassays were calculated from probit regressions using the computer program POLO-PC (LeOra Software, Berkely, USA).

3 RESULTS AND DISCUSSION

3.1 Biological efficacy of imidacloprid metabolites

Three of the selected metabolites of imidacloprid (Fig 1) were active against SUD-S, the susceptible reference strain of *B. tabaci*, in oral ingestion bioassays using artificial double membranes (Table 1). The olefine metabolite **1** was approximately ten times more active than the parent compound, imidacloprid. The 4-hydroxy metabolite **2** was as active as imidacloprid, while the 5-hydroxy metabolite **3** was less active than the parent compound. The dihydroxy compound **4** and the urea metabolite **5** had no effect in sachet tests at up to 60 mg litre^{-1}. These results were quite consistent with those already published for the aphid species *Myzus persicae* Sulz. and *Aphis gossypii* Glov.[11] Both hydroxy metabolites (**2** and **3**) were also tested on the insecticide-resistant strains of *B. tabaci*. In those bioassays the 5-hydroxy metabolite **3** was at least 15 times less active than the 4-hydroxy compound **2** against all strains tested (Table 2). The responses of the two strains from

Table 1. Efficacy of plant metabolites of imidacloprid against an insecticide-susceptible strain (SUD-S) of *Bemisia tabaci* in oral ingestion bioassays (48 h)

Compound	LC_{50} (*mg litre*$^{-1}$)	*FL 95%*	*Slope*
Imidacloprid	0.24	0.064–0.59	1.50
1	0.025	0.017–0.032	1.61
2	0.15	0.039–0.32	1.60
3	2.4	0.67–7.2	1.53
4	>60	—	—
5	>60	—	—

Compound	Strain	LC_{50} (mg litre^{-1})	FL 95%	Slope	RR[a]
2	SUD-S	0.15	0.039–0.32	1.60	–
	ALM-2	0.55	0.15–1.3	1.22	4
	LMPA-2	0.27	0.059–0.72	1.18	2
	CAL-1	0.10	0.049–0.17	2.29	1
3	SUD-S	2.4	0.67–7.2	1.53	–
	ALM-2	12	4.2–31	1.69	5
	LMPA-2	5.1	0.44–13	1.21	2
	CAL-1	2.7	0.34–11	0.910	1

[a] Response relative to susceptible strain.

Table 2. Efficacy of metabolites **2** (4-OH-imidacloprid) and **3** (5-OH-imidacloprid) against different insecticide-resistant strains of *Bemisia tabaci* in oral ingestion bioassays (48 h)

Almeria, Spain, were two to five times lower than that of the susceptible strain SUD-S, but not significantly different (Table 2).

3.2 Physicochemical properties

The log $P_{o/w}$ values of all metabolites tested were comparable to that of imidacloprid (Table 3), indicating a hydrophilic character and suggesting that they could be as (xylem-) mobile as imidacloprid within the plant. The very active olefine metabolite **1** was the only compound with an acidic proton. It has a pK_a of 7.2 and a log $P_{o/w}$ of 0.45 and 0.85 at pH 7.5 and pH 2.1, respectively (Table 3). According to the model of Kleier,[19] these physicochemical properties should enable the compound to be translocated in the phloem. Neither imidacloprid nor the other metabolites (**2**–**5**) showed any acidic properties, and it has been shown in previous studies that imidacloprid, at least, is virtually non-phloem-mobile.[7,8]

3.3 Uptake and translocation of imidacloprid and the olefine metabolite 1

3.3.1 Recovery and total uptake

Recovery of the total applied radioactivity was 98–102% after combustion in an oxidiser. The efficiency of extraction of the radioactivity from the plant matrix was 98% for [^{14}C]imidacloprid and 96% for the ^{14}C-olefine compound **1**.

Since investigation of the comparative translocation of imidacloprid and the olefine metabolite **1** was the primary objective, it was important to ensure that a measurable, and more or less equal, amount of both compounds was taken up into the leaf. The adjuvant Silwet L-77 was used to increase leaf uptake, because it allows infiltration of the stomata, and a quick mass flow of the liquid phase which is quite different from normal penetration via diffusion through a limiting skin.[20] Leaf uptake of imidacloprid and metabolite **1** were 35% and 32% of the recovered activity, respectively.

3.3.2 Stability on the leaf surface

[^{14}C]Imidacloprid was quite stable on the leaf surface: of the material remaining on the surface, 88% was parent compound and 3.5% the olefine metabolite **1**. However, on leaves treated with ^{14}C-olefine metabolite **1**, only 38% of the remaining activity was present as unchanged material. Since this compound is stable in solution, it suggests that it is unstable on the leaf surface, an unexpected result. Although cotton leaves can have surface pH values as high as pH 9,[21] metabolite **1** has a half life of >250 h at this pH (Table 3). The compound may be very sensitive to photodegradation, but this aspect was not investigated.

3.3.3 Distribution of radioactivity within the plant

To obtain more detailed information about the uptake and translocation of both compounds, autoradiographs were prepared, with subsequent combustion analysis of different plant fractions (Table 4). Autoradiographs showed that the silicone seal around the zone of application was effective (data not shown). Therefore, all information about translocation is based on transport within the plant.

For both imidacloprid and metabolite **1**, the major part of the radioactivity taken up into the plant remained at the site of application and of the fraction of radioactivity that was translocated, the major portion moved acropetally, 27.9 and 28.8%, respec-

Compound	$logP_{o/w}$ pH 7.5	$LogP_{o/w}$ pH 2	pKa	Half-life (h) pH 7	Half-life (h) pH 9
Imidacloprid	1.07	1.07	—	>250	>250
1	0.45	0.85	7.20	>250	>250
2	0.80	0.88	—	nd	nd
3	0.80	0.88	—	nd	nd
4	0.69	0.78	—	nd	nd
5	0.86	0.89	—	nd	nd

Table 3. Physicochemical properties of imidacloprid and selected metabolites

Table 4. Distribution of radioactivity[a] in cotton plants 26 h after foliar application of [^{14}C]imidacloprid and ^{14}C-olefine metabolite **1**

Fraction	*Imidacloprid*		*Olefine*	
	(% *of recovery*)[c]	*SD*[b]	(% *of recovery*)[c]	*SD*[b]
Leaf surface wash	65.0	2.56	67.7	5.28
Application site after washing	27.9	2.48	28.8	4.92
Leaf above application[d] site	5.12	0.10	2.94	0.42
Cotyledons[e]	0.05	0.02	0.03	0.01
2nd leaf[e]	0.18	0.05	0.06	0.01
Shoot tip[e]	0.20	0.05	0.05	0.02
Stem	1.46	0.18	0.47	0.10
Root[e]	0.09	0.03	0.03	0.00

[a] All values determined by combustion in an oxidiser, except those for washing from the leaf surface.
[b] Standard deviation, $n = 3$.
[c] Total recovery of applied radioactivity was 98–102%.
[d] Equals the amount of radioactivity which was translocated acropetally.
[e] Radioactivity which was translocated basipetally from the treated leaf.

tively. There was no significant difference between metabolite **1** and imidacloprid with regard to phloem (basipetal) mobility. Preliminary experiments with imidacloprid showed that basipetal translocation of radioactivity was extremely low in, eg, wheat, cabbage and apple, and that acropetal translocation after leaf application was 10–100 times higher than basipetal translocation, at least within the 24–48 h period tested (ReckmannU, unpublished). Our present results indicate that the distribution of total radioactivity is probably not a suitable basis for comparing the translocation of these compounds in cotton after leaf application, because both compounds were metabolised rapidly.

*3.3.4 Identification of [^{14}C]imidacloprid and ^{14}C-olefine compound **1** in plant extracts*

Long-term experiments have already shown that imidacloprid is metabolised much faster in cotton than in wheat.[7,8] Extraction and TLC of the different plant fractions in the present short-term experiment revealed that imidacloprid and metabolite **1** were both metabolised rapidly in cotton (Tables 5 and 6). Twenty-six hours after application, 32% and 43% of the radioactivity within the plant could be identified as parent compound for [^{14}C]imidacloprid and ^{14}C-olefine metabolite **1,** respectively. However, there was great variability in the distribution of parent compound between different plant fractions. The bulk of radioactivity within the plant could be extracted from the site of application.

Basipetal from the point of application, only 5.6% of the extractable radioactivity could be identified as parent compound for [^{14}C]imidacloprid, but 24.4% for ^{14}C-olefine metabolite **1.** However, if these portions are compared with the total amount of radioactivity taken up into the plant, the basipetal fraction shows only a minor difference between imidacloprid and **1,** for which 0.54% and 0.76%, respectively, of total radioactivity could be detected. Similar amounts of imidacloprid and **1** were also recovered in an acropetal direction. Combining the data from Tables 2 and 3 shows that 2.1% and 2.4% of the radioactivity which was taken up corresponded to acropetally translocated parent [^{14}C]imidacloprid and ^{4}C-olefine metabolite **1,** respectively.

The main part of the radioactivity which had been translocated away from the site of application of both labelled imidacloprid and metabolite could not be identified as parent compound. Although only a small fraction of applied [^{14}C]imidacloprid was recovered as ^{14}C-metabolite **1** (Table 2), it might

Table 5. Distribution of radioactivity in extracts of cotton plants 26 h after application of [^{14}C]imidacloprid: Identification of [^{14}C]imidacloprid and ^{14}C-olefine metabolite **1**

Extracted plant fraction	*Radioactivity of total plant extract* (% *of total extract*)	*Identified as* [^{14}*C*] *imidacloprid* (% *of fraction*)	*Identified as* ^{14}*C-olefine metabolite* 1 (% *of fraction*)
Application site after washing[a]	77.0	37.4	4.7
Leaf above application[b] site	13.3	16.1	4.9
Rest of the plant[c]	9.7	5.6	1.2

[a] Washing with acetonitrile removed 65% of applied activity.
[b] Acropetal translocation of radioactivity.
[c] Basipetal translocation of radioactivity.

Table 6. Distribution of radioactivity in extracts of cotton plants 26 h after application of [^{14}C]olefine metabolite **1**: Identification of ^{14}C-olefine metabolite **1** and [^{14}C]imidacloprid

Extracted plant fraction	*Radioactivity of total plant extract (% of total extract)*	*Identified as ^{14}C-olefine metabolite* **1** *(% of fraction)*	*Identified as [^{14}C] imidacloprid (% of fraction)*
Application site after washing[a]	88.6	45.4	—
Leaf above application[b] site	8.3	28.8	—
Rest of the plant[c]	3.1	24.4	—

[a] Washing with acetonitrile removed 68% of applied activity.
[b] Acropetal translocation of radioactivity.
[c] Basipetal translocation of radioactivity.

nevertheless play an important role in whitefly control, due to its higher activity as measured in oral ingestion bioassays. Most of the radioactivity detected away from the site of application was related to several slower-moving, less active, metabolites which are more polar than imidacloprid and metabolite **1,** as revealed by TLC analysis.

4 CONCLUSIONS

Three of the five *in planta* metabolites of imidacloprid selected for this study were active against the cotton whitefly *B. tabaci* in oral ingestion bioassays. The olefine metabolite **1** was *c*10 times more active than the parent compound imidacloprid, whereas activity of the 4-hydroxy metabolite **2** was comparable to that of imidacloprid and that of the 5-hydroxy compound **3** was inferior. The physicochemical properties of the olefine metabolite **1** were different from those of imidacloprid. Although metabolite **1** contains an acidic proton (pKa 7.2), the results indicated that it does not differ from imidacloprid in short-term (26 h) translocation (acropetal and basipetal) in cotton plants. However, some of the metabolites occuring in plants after seed treatment, soil application and even foliar application were active against whiteflies, and they might protect the plant as it grows, despite decreasing concentrations of imidacloprid itself.

ACKNOWLEDGEMENTS

The excellent technical assistance of Mrs Bungert is gratefully acknowledged. The ^{14}C-olefine metabolite of imidacloprid was kindly provided by Mrs Klempner.

REFERENCES

1 Elbert A, Becker B, Hartwig J and Erdelen C, Imidacloprid–a new systemic insecticide. *Pflanzenschutz Nachrichten Bayer* **44:**113–136 (1991).
2 Nauen R, Strobel J, Tietjen K, Otsu Y, Erdelen C and Elbert A, Aphicidal activity of imidacloprid against a tobacco feeding strain of *Myzus persicae* (Homoptera: Aphididae) from Japan closely related to *Myzus nicotianae* and highly resistant to carbamates and organophosphates. *Bull Entomol Res* **86:**165–171 (1996).
3 Elbert A, Nauen R, Cahill M, Devonshire AL, Scarr AW, Sone S and Steffens. R, Resistance management with chloronicotinyl insecticides using imidacloprid as an example. *Pflanzenschutz Nachrichten Bayer* **49:**5–54 (1996).
4 Bai D, Lummis SCR, Leicht, W, Breer H and Sattelle DB, Actions of imidacloprid and a related nitromethylene on cholinergic receptors of an identified insect motor neurone. *Pestic Sci* **33:**197–204 (1991).
5 Liu M-Y and Casida JE., High-affinity binding of [^{3}H]imidacloprid in the insect acetylcholine receptor. *Pestic Biochem Physiol* **46:**40–46 (1993).
6 Nauen R and Elbert A, Effect of imidacloprid on aphids after seed treatment of cotton in laboratory and greenhouse experiments. *Pfanzenschutz Nachrichten Bayer* **47:**181–216 (1994).
7 Stein-Dönecke. U, Führ F, Wienecke. J, Hartwig J and Leicht W, Influence of soil moisture on the formation of dressing zones and uptake of imidacloprid after seed treatment of winter wheat. *Pfanzenschutz Nachrichten Bayer* **45:**327–368 (1992).
8 Tröltzsch CM, Führ F, Wieneke J and Elbert A, Einfluß unterschiedlicher Bewässerungsverfahren auf die Aufnahme von Imidacloprid durch Baumwolle nach Saatgutbeizung. *Pfanzenschutz Nachrichten Bayer* **47:**249–303 (1994).
9 Araki Y, Bornatsch W, Brauner A, Clark T, Dräger G, Kurogochi S, Sakamoto H and Vogeler K, Metabolism of imidacloprid in plants. *Proc IUPAC Congress, Washington* 2B–157 (1994).
10 Anonymous, Metabolism. *Nihon Noyaku Gakkaishi* (*J Pestic Sci*) *Special Issue* **19:**301–306 (1994).
11 Nauen R, Tietjen K, Wagner K and Elbert A, Efficacy of plant metabolites of imidacloprid against *Myzus persicae* and *Aphis gossypii* (Homoptera: Aphididae). *Pestic Sci* **52:**53–57 (1998).
12 Westwood F, Bean KM, Dewar AM, Bromilow RH and Chamberlain K, Movement and presistence of [^{14}C]imidacloprid in sugar-beet plants following application to pelleted sugar-beet seed. *Pestic Sci* **52:**97–104 (1998).
13 Köster J, Comparative metabolism of [*pyridyl-*^{14}C-*methyl*] imidacloprid in plant cell suspension cultures. *Proc Brighton Crop Protect Conf–Pests and Diseases* **2:**901–906 (1992).
14 Cahill M, Gorman K, Day S, Denholm I, Elbert A. and Nauen R, Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bull Entomol Res* **86:**343–349 (1996).
15 Elbert A and Nauen R, Bioassays for imidacloprid for a resistance monitoring against the whitefly *Bemisia tabaci. Proc. Brighton Crop Protect Conf–Pests and Diseases* 6D-**8:**731–738 (1996).
16 Nauen R, Koob B, Klüver T and Elbert A, Biochemical characterization of insecticide-resistant strains of the tobacco

whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). *Mitt Dtsch Ges Allg Angew Ent* **11**:217–221 (1997).
17 Amtsblatt EG Nr. L 383 A/63 (A.8 Verteilungskoeffizient).
18 Cleveland JA, Benko JR, Benko MH, Gluck SJ and Walbroehl YM, Automated pKa determination at low solute concentrations by capillary electrophoresis. *J Chromatogr* **652**:301–308 (1993).
19 Kleier DA, Phloem mobility of xenobiotics. *Plant Physiol* **86**:803–810 (1988).
20 Stevens PJG, Organosilicone surfactants as adjuvants for agrochemicals. *Pestic Sci* **38**:103–122 (1993).
21 Harr J and Guggenheim R (eds), *The Leaf Surface of Major Crops*, Friedrich Reinhart. pp 44–46 (1995).